RESEARCH PAPER | Botany | Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

Diversity of Plant Growth Promotory Rhizobacteria From Maize Rhizosphere

KEYWORDS: Rhizosphere, Plant growth promotory rhizobacteria, Pseudomonas, Klebsiella.

Seema Rawat

Department of Botany and Microbiology, H.N.B. Garhwal (Central) University, Srinagar, Uttarakhand, India

ABSTRACT *The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. It harbours a wide range of bacteria influenced by the root exudates. These bacteria can be beneficial or deleterious. The present work was focused on characterization of plant growth promotory rhizobacteria from maize rhizosphere of three fields. The maize rhizobacterial population (log10cfu) varied significantly in all the three fields from 5.4 to 9.5. In field A, Alcaligenes was documented to be dominant population of zero day (40%). Klebsiella was the dominnat rhizobacteria on 30th day (40%) while Pseudomonas and Alcaligenes were equally dominant on 60th day (30%). Pseudomonas was dominant on 90th day (60%) as well as 120th day (47%). In field B Alcaligenes was the dominant isolate on zero day (40%) while Alcaligenes and Klebsiella were dominant on 30th day (30%). Pseudomonas was dominant on 60th day (40%) and on 90th day (55%) and on 120th day (55%). In field C, Corynebacterium was dominant isolate (40%) on zero day while Alcaligenes was dominant on 30th day (40%), Klebsiella on 60th day (40%), Pseudomonas on 90th day (65%) and on 120th day (40%). The recovered population showed good growth promotion attributes viz., phosphorus solubilization, rhamnolipid, siderophore and protease production. Corynebacterium, Klebsiella and Pseudomonas were found to promote length of shoot in sterile as well as non-sterile soil after 30days and 45 days of sowing.*

Introduction

Maize (*Zea mays* L) is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. In India, maize is the third most important food crops after rice and wheat. Maize can be grown successfully in variety of soils ranging from loamy sand to clay loam. However, soils with good organic matter content having high water holding capacity with neutral pH are considered good for higher productivity. The pressure of producing high yields of food crops has led to extensive use of fertilizers which has adversely affected the soil health. Therefore the global interest has shifted towards adopting organic farming using plant growth promoting rhizobacteria as biofertilizers rather than chemical fertilizers. Thus it necessitates to have an understanding of interactions between rhizosphere and bacteria residing there so that they can be used as bioinoculants. PGPRs are from diverse genera like *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Corynebacterium*, *Pseudomonas*, *Rhizobium*, *Serratia* etc, of which *Bacillus* and *Pseudomonas spp.* are predominant (Glick, 1995; Podile & Koshore, 2007). The present work was aimed at characterization of rhizobacteria from maize rhizosphere

2. Materials and Methods

2.1 Collection of samples

Samples were collected from three different fields (Field A located in Manduwala; Field B located in Tilwadi and Field C located in Bahuwala) at different time periods viz., 0d, 30d, 60d, 90d and 120d.

2.2 Physical characteristics of soil

The temperature and pH of soil sample was recorded.

2.3 Recovery of rhizospheric microflora

Rhizospheic soil was separated from roots of maize with the help of brush in a petridish. 10g soil was placed in 100ml sterile Phosphate buffered saline (PBS) and was placed in shaker for 1h. 0.1 ml of appropriately diluted sample was spreaded on nutrient agar. All fractions were plated in triplicates. Plates were incubated in a BOD incubator at 28±1^{0}C for 24.

2.4 Characterization of isolates

2.4.1 Morphological characterization

Morphological characteristics viz., colony morphology (colour, chromogenesis, shape, margin, elevation and surface) and cell morphology (shape, gram reaction and arrangement) of recovered isolates were studied.

2.4.2 Biochemical characterization

The various biochemical characteristics viz., Oxidase test, IMViC test, TSI test, Uresae test, Catalase test and nitrate reduction test were carried out according to Cappucino and Sherman (1992).

2.4.3 Functional characterization

The functional diversity amongst recovered isolates was studied by qualitative screening of their ability to solubilize phosphorus, protease, rhamnolipid and siderophore production.

(a) Phosphorus solubilization

Isolates exhibiting clearing zone on Pikovaskya's agar after 96-120h of incubation were considered as positive.

(b) Rhamnolipid production- It was estimated according to Sharma and Johri (2002). All isolates were inoculated on rhamnolipid production medium. Isolates exhibiting blue colour were considered as positive.

(c) Siderophore production- It was assayed according to Schwyne and Neilands (1987). Isolates were spot inoculated on Chromeazurol 'S' agar. Isolates exhibiting an orange halo zone after 48-72h of incubation were considered positive. Their zone diamter was measured.

(d)Protease production- It was assayed on skim milk agar. Isolates exhibiting a clear halo zone after 24h of incubation were considered as positive. Their zone diameter was measured.

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

2.4.4 Effect of rhizobacterial preinoculation on plant growth

The promising isolates were tested for their effect on plant growth by dipping seeds in overnight grown broth culture and sowing in sterilized soil and non-sterilized soil in pots in polyhouse.

3. Results

Temperature of soil sample of maize varied significantly from 38±0.45^{0}C (0d) to 24±0.32^{0}C (120d). pH varied slightly from 7.1 (0d) to 6.7(120d).

3.1 Diversity of rhizobacteria

3.1.1 Structural diversity

The maize rhizobacterial population (log_{10}cfu) varied significantly in all the three fields from 5.4 to 9.5 (Fig. 1). In field A, *Alcaligenes* was documented to be dominant population of zero day (40%) (Fig.2). *Klebsiella* was the dominnat rhizobacteria on 30th day (40%) while *Pseudomonas* and *Alcaligenes* were equally dominant on 60th day (30%). *Pseudomonas* was dominant on 90th day (60%) as well as 120th day (47%) (Fig. 2). In field B *Alcaligenes* was the dominant isolate on zero day (40%) while *Alcaligenes* and *Klebsiella* were dominant on 30th day (30%). *Pseudomonas* was dominant on 60th day (40%) and on 90th day (55%) and on 120th day (55%) (Fig. 3). In field C, *Corynebacterium* was dominant isolate (40%) on zero day while *Alcaligenes* was dominant on 30th day (40%), *Klebsiella* on 60th day (40%), *Pseudomonas* on 90th day (65%) and on 120th day (40%) (Fig. 4).

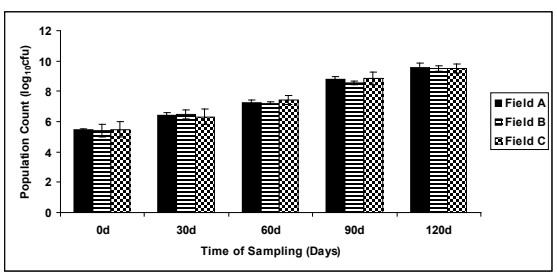
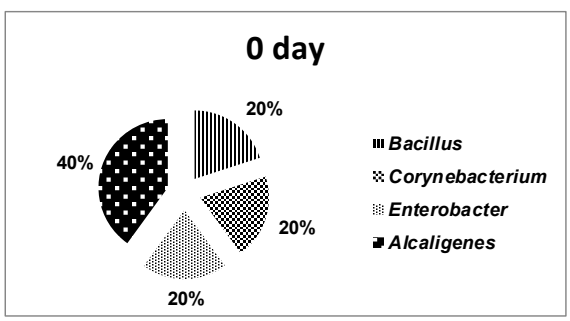
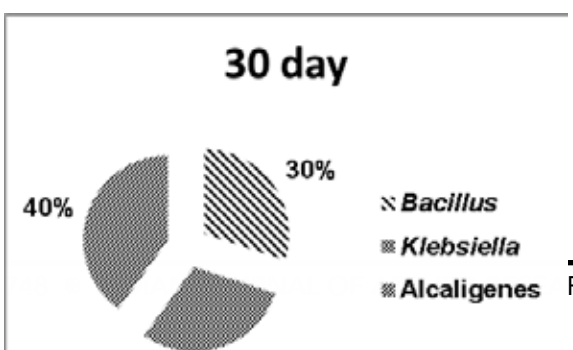
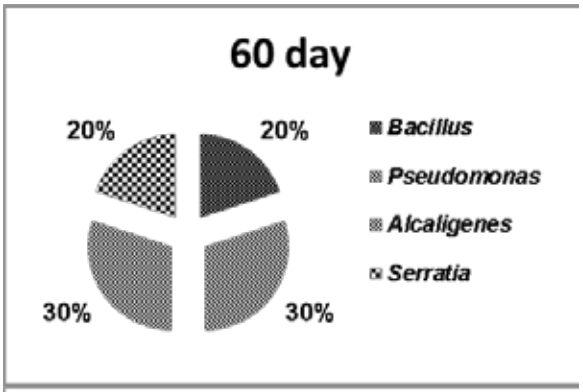
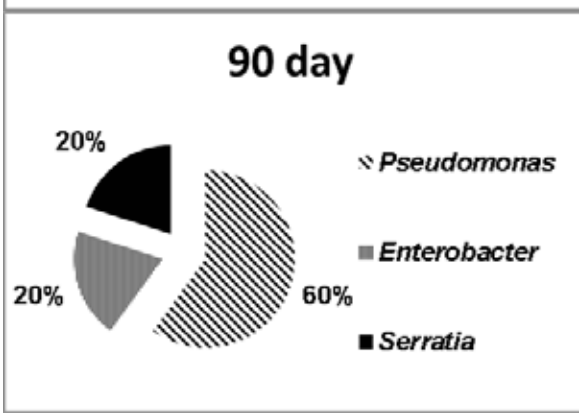


Fig. 1: Population structure of maize rhizobacteria









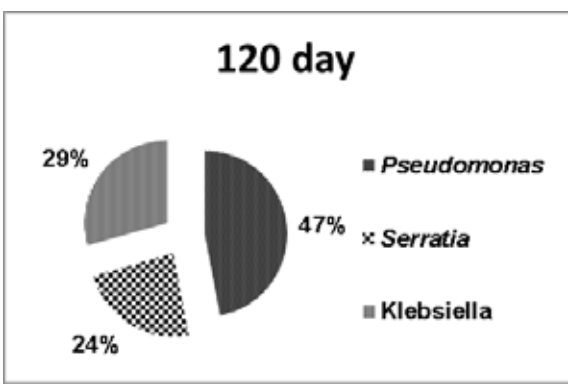
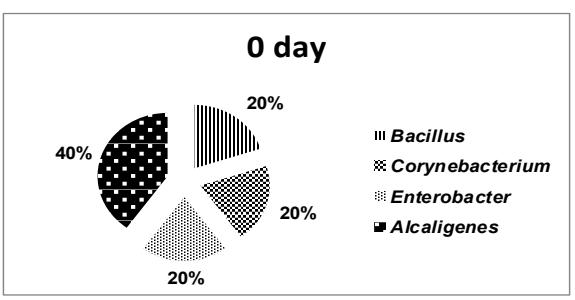
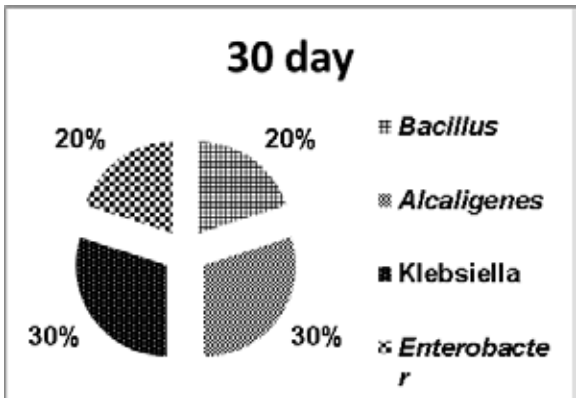
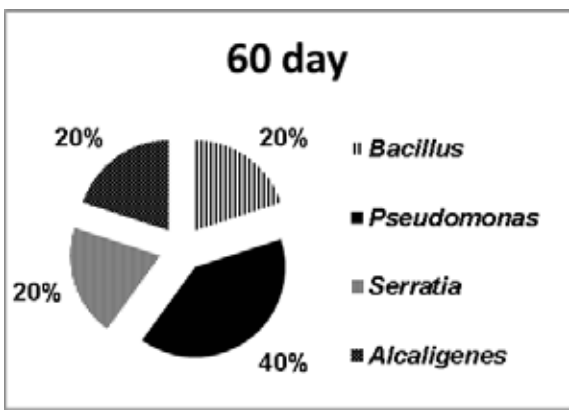


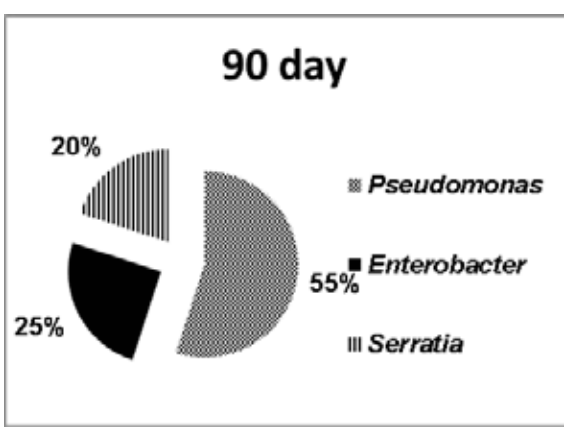
Fig. 2: Distribution of rhizobacteria in different days of maize crop in field A

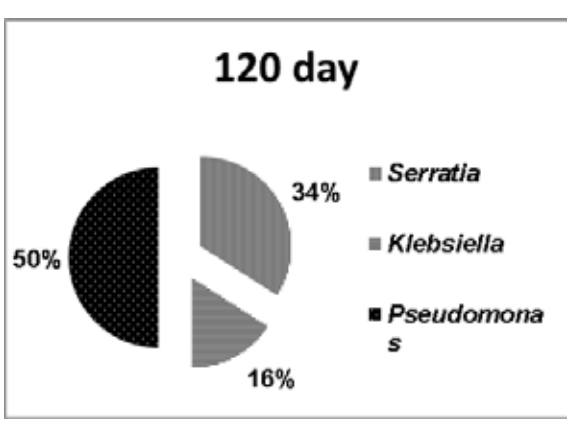


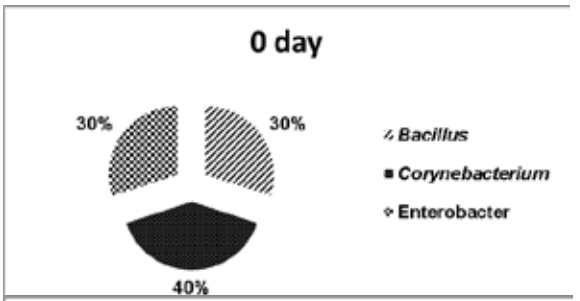
Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

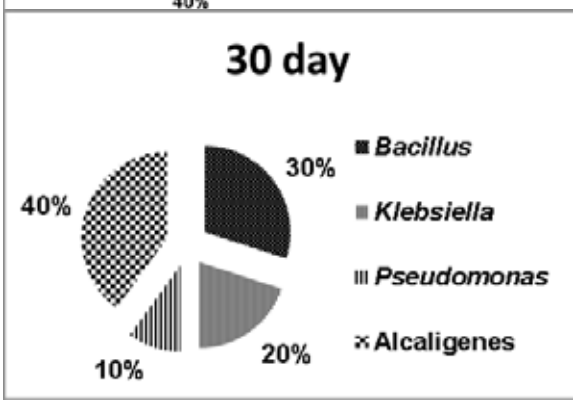


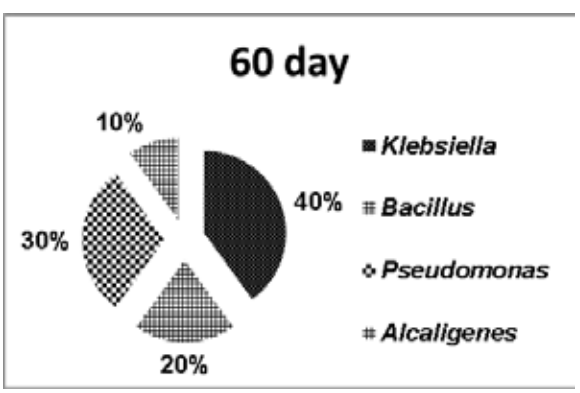












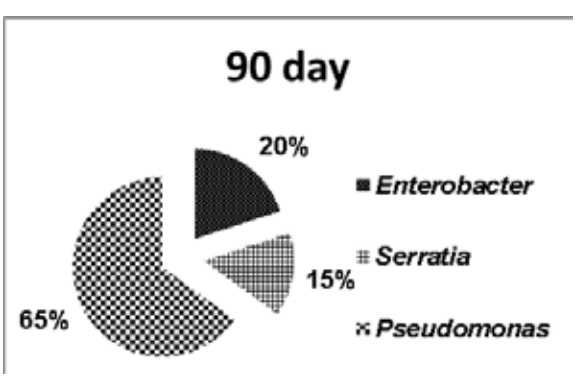


Fig. 3: Distribution of rhizobacteria in different days of maize crop in field B

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

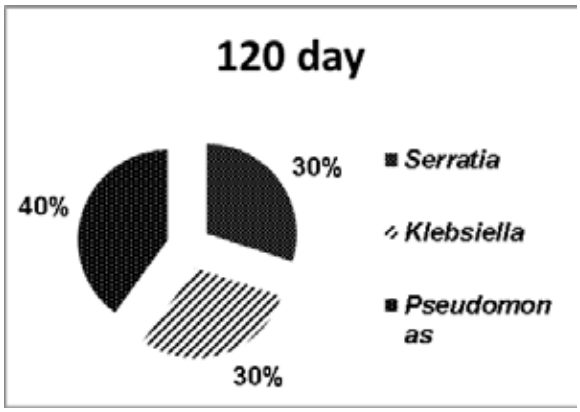


Fig. 4: Distribution of rhizobacteria in different days of maize crop in field C

3.1.2 Functional diversity of rhizobacteria

The distributional of functional diversity amongst the recovered rhizobacteria is depicted in Fig. 5. Maximum siderophore producers were recovered from 30d, 60d and 90d samples. Maximum P solubilizers were recovered from 60d sample while maximum protease producers were recovered from 60d sample. Maximum rhamnolipid producers were recovered from 60d and 90d samples.

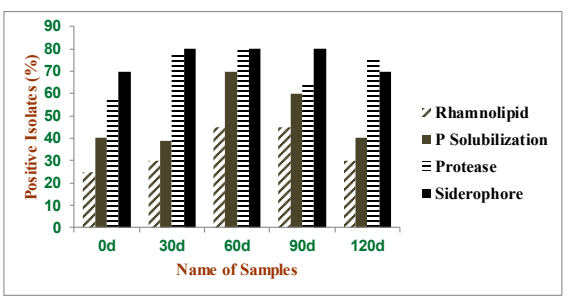


Fig. 5: Functional diversity of recovered rhizobacteria from maize rhizosphere

3.2 Testing of growth promotion potential of rhizobacteria

Corynebacterium, *Klebsiella* and *Pseudomonas* were found to promote length of shoot in sterile as well as non sterile soil after 30days and 45 days of sowing (Table 1).

Table 1: Effect of preinoculation of seeds with rhizobacteria on shoot length of maize plants

	Length of shoot after 30 days of sowing (cm)		Length of shoot after 45 days of sowing (cm)	
Isolate	In non-sterile soil	In sterile soil	In non-sterile soil	In sterile soil
Control	28.0	25.0	38.0	35.0
Bacillus	18.5	14.5	26.5	24.5
Enterobacter	20.5	16.0	30.0	28.0
Corynebacterium	29.7	27.1	38.7	37.8
Klebsiella	29.2	25.0	39.6	35.0
Pseudomonas	35.0	30.0	46.0	41.0
Alcaligenes	25.7	22.0	34.0	30.5
Serratia	23.0	18.5	31.0	27.0

4. Discussion

The intensive use of fertilizers has led to adverse effect on soil health. The population profile of rhizobacteria was observed to change with the age of crops. Rhizobacterial population (log_{10} cfu) varied from 5.4 to 9.5. The distribution of rhizobacteria varied with the age of crops. *Pseudomonas* and *Alcaligenes* were documented to be dominant rhizobacteria from three fields. The distribution pattern of rhizobacteria in all the three fields was different. However the distribution and dominance pattern of rhizobacteria is usually influenced by the crop but there could be many factors influencing it like variety of crop grown, soil health etc. The functional diversity was also observed to be influenced with the age of crop as siderophore producers were maximum on 30d, 60d and 90d while maximum P solubilizers and protease producers were from 60d of crop. Maximum rhamnolipid producers were from 60d and 90d crops. *Bacillus*, *Klebsiella* and *Pseudomonas* species were documented to be strong siderophore producers as well as phosphate solubilizers. *Corynebacterium* species showed strong protease producing ability. Siderophores improve plant growth and development by increasing the accessibility of iron in the soil surrounding the roots (Kloepper *et al*., 1980; Marschner & Romheld, 1994). *Bacillus*, *Klebsiella*, and *Pseudomonas* are some of the genera that produce siderophores.

Phosphorus, which is taken by the plants from soil as phosphate anions, is necessary for plant growth. But the amount available to plants is very low because of its extreme insolubility. Rhizobacteria solubilize phosphate by secreting some acids or by some other means and these bacteria are collectively termed as phosphate solubilizing bacteria- PSB (de Freitas *et al*., 1997; Rodriguez and Fraga, 1999; Nautiyal *et al*., 2000; Chen *et al*., 2006). Several researchers have consequently proven that PSB increase plant growth and yield (Griener & Larsson, 2001; Moura *et al*., 2001). A deeper understanding of rhizopshere and bacteria is essential to develop promising isolates as bioinoculants. The promising isolates were tested for *in vitro* growth promotion. *Corynebacterium*, *Klebsiella* and *Pseudomonas* were found to promote length of shoot in sterile as well as non-sterile soil after 30days and 45 days of sowing. This study resulted in some promising isolates which can be used as biofertilizers to enhance crop production but further fields trials are required for commercialization of these biofertilizers.

Volume : 5 | Issue : 5 | May 2015 | ISSN - 2249-555X

REFERENCE

1. Cappucinno, J.G. and Sherman, N. (1992). Microbiology: a laboratory manual. The Benjamin/Cummings Publishing Co, USA, 3rd ed. pp: 150. | 2. Chen Y.P., Rekha P.D., Arun A.B., Shen F.T., Lai W.A., Young C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities, Appl Soil Ecol., 34: 33–41. | 3. de Freitas J.R., Banerjee M.R., Germida J.J. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus). Biol. Fertil. Soils, 24: 358-364. | 4. Glick B.R. (1995) The enhancement of plant growth by free-living bacteria. Can J. Microbiol 41:109-117. | 5. Greiner R. and Larsson Alminger M. 2001. Stereospecificity of myo-inositol hexakisphosphate dephosphorylation by phytatedegrading enzymes of cereals J Food Biochem., 25: 229-248. | 6. Kloepper J.W., Leong J., Teintze M., Schroth M.N. 1980. Pseudomonas siderophores: a mechanism explaining disease suppressive soils. Current Microbiol., 4: 317-320. | 7. Marschner H. and Rohmeld V. 1994. Strategies of plants for acquisition of iron. Plant Soil, 165:261-274. | 8. Moura D.S., Bergey D.R., Ryan C.A. 2001. Characterization and localization of a wound-inducible type I serine carboxypeptidase from leaves of tomato plants (Lycopersicon esculentum Mill.). Planta, 212: 222–230. | 9. Nautiyal C.S., Bhadauria S., Kumar P., Lal H., Mondal R. and Verma R. 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils, FEMS Microbiol. Lett., 182: 291-296. | 10. Podile, A.R. and Kishore, K. 2007. Plant growth-promoting rhizobacteria. In: Plant associated bacteria. Springer Netherlands, pp. 195–230. | 11. Rodriguez H. and Fraga R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv., 17: 319-339. | 12. Schwyne, B. and Neilands, J.B. 1987. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem., 160: 40-47. | 13. Sharma, A. and Johri, B.N. 2003. Combat of iron-deprivation through a plant growth promoting fluorescent Pseudomonas strain GRP3 in mung bean. Microb. Res., 158: 1-5. |